Claims

1. A method of making a glycoconjugate comprising a saccharide conjugated to a carrier protein through a (2-((2-oxoethyl)thio)ethyl)carbamate (eTEC) spacer, comprising the steps of:

a) reacting a saccharide with 1,1'-carbonyl-di-(1,2,4-triazole) (CDT) or 1,1'-carbonyldiimidazole (CDI), in an organic solvent to produce an activated saccharide;

b) reacting the activated saccharide with cystamine or cysteamine or a salt thereof, to produce a thiolated saccharide;

c) reacting the thiolated saccharide with a reducing agent to produce an activated thiolated saccharide comprising one or more free sulfhydryl residues;

d) reacting the activated thiolated saccharide with an activated carrier protein comprising one or more α-haloacetamide groups, to produce a thiolated saccharide-carrier protein conjugate; and

e) reacting the thiolated saccharide-carrier protein conjugate with (i) a first capping reagent capable of capping unconjugated α-haloacetamide groups of the activated carrier protein; and/or (ii) a second capping reagent capable of capping unconjugated free sulfhydryl residues;

whereby an eTEC linked glycoconjugate is produced.

2. The method of claim 1, wherein the capping step e) comprises reacting the thiolated saccharide-carrier protein conjugate with (i) N-acetyl-L-cysteine as a first capping reagent, and/or (ii) iodoacetamide as a second capping reagent.

3. The method of claim 1 or 2, further comprising a step of compounding the saccharide by reaction with triazole or imidazole to provide a compounded saccharide, wherein the compounded saccharide is shell frozen, lyophilized and reconstituted in an organic solvent prior to step a).

4. The method of claim 1, 2 or 3, further comprising purification of the thiolated polysaccharide produced in step c), wherein the purification step comprises diafiltration.

5. The method of any one of claims 1 to 4, wherein the reducing agent in step c) is tris(-2-carboxyethyl)phosphine (TCEP), dithiothreitol (DTT) or mercaptoethanol.

6. The method of any one of claims 1 to 5, wherein the carrier protein is activated with an activated bromoacetic acid derivative.

7. The method of claim 6, wherein the bromoacetic acid derivative is the N-hydroxysuccinimide ester of bromoacetic acid (BAANS).

8. The method of any one of claims 1 to 7, wherein the method further comprises purification of the eTEC linked glycoconjugate by diafiltration.

9. The method of any one of claims 1 to 8, wherein the organic solvent in step a) is a polar aprotic solvent selected from the group consisting of dimethyl sulfoxide (DMSO), dimethylformamide (DMF), dimethylacetamide (DMA), *N*-methyl-2-pyrrolidone (NMP), acetonitrile, 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) and hexamethylphosphoramide (HMPA), or a mixture thereof.

10. The method of any one of claims 1 to 9, wherein the saccharide : carrier protein ratio (w/w) is between 0.2 and 4.

11. The method of claim 10, wherein the saccharide : carrier protein ratio (w/w) is between 0.4 and 1.7.

12. The method of any one of claims 1 to 11, wherein the saccharide is a capsular polysaccharide derived from *S. pneumoniae*.

13. The method of claim 12, wherein the capsular polysaccharide is selected from the group consisting of pneumococcal (Pn) serotype 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F capsular polysaccharides.

14. The method of claim 13, wherein the capsular polysaccharide is a Pn-serotype 33F capsular polysaccharide.

15. The method of claim 13, wherein the capsular polysaccharide is a Pn-serotype 22F capsular polysaccharide.

16. The method of claim 13, wherein the capsular polysaccharide is a Pn-serotype 10A capsular polysaccharide.

17. The method of claim 13, wherein the capsular polysaccharide is a Pn-serotype 11A capsular polysaccharide.

18. The method of any one of claims 1 to 11, wherein the saccharide is a capsular polysaccharide derived from *N. meningitidis*.

19. The glycoconjugate of claim 19, wherein the capsular polysaccharide is selected from the group consisting of meningococcal (Mn)-serotype A, C, W135 and Y capsular polysaccharides.

20. The method of any one of claims 1-19, wherein the carrier protein is CRM_{197}.

21. A glycoconjugate produced by the method of any one of claims 1-20.

22. An immunogenic composition comprising the glycoconjugate of claim 21 and a pharmaceutically acceptable excipient, carrier or diluent.

23. A glycoconjugate comprising a bacterial capsular polysaccharide conjugated to a carrier protein through an eTEC spacer, wherein the polysaccharide is covalently linked to the eTEC spacer through a carbamate linkage, and wherein the carrier protein is covalently linked to the eTEC spacer through an amide linkage.

24. The glycoconjugate of claim 23, wherein the capsular polysaccharide is derived from *S. pneumoniae*.

25. The glycoconjugate of claim 24, wherein the capsular polysaccharide is selected from the group consisting of Pn-serotype 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F capsular polysaccharides.

26. The glycoconjugate of claim 25, wherein the capsular polysaccharide is a Pn-serotype 33F capsular polysaccharide.

27. The glycoconjugate of claim 25, wherein the capsular polysaccharide is a Pn-serotype 22F capsular polysaccharide.

28. The glycoconjugate of claim 25, wherein the capsular polysaccharide is a Pn-serotype 10A capsular polysaccharide.

29. The glycoconjugate of claim 25, wherein the capsular polysaccharide is a Pn-serotype 11A capsular polysaccharide.

30. The glycoconjugate of claim 23, wherein the capsular polysaccharide is derived from *N. meningitidis*.

31. The glycoconjugate of claim 30, wherein the capsular polysaccharide is selected from the group consisting of Mn-serotype A, C, W135 and Y capsular polysaccharides.

32. The glycoconjugate of any one of claims 23 to 31, wherein the polysaccharide has a molecular weight of between 10 kDa and 2,000 kDa.

33. The glycoconjugate of claim 32, wherein the polysaccharide has a molecular weight of between 50 kDa and 2,000 kDa.

34. The glycoconjugate of any one of claims 23 to 33, wherein the glycoconjugate has a molecular weight of between 50 kDa and 20,000 kDa.

35. The glycoconjugate of claim 34, wherein the glycoconjugate has a molecular weight of between 500 kDa and 10,000 kDa.

36. The glycoconjugate of any one of claims 23 to 35, wherein the polysaccharide has a degree of O-acetylation between 75-100%.

37. The glycoconjugate of any one of claims 23 to 36, wherein the carrier protein is CRM_{197}.

38. The glycoconjugate of claim 37, wherein the CRM_{197} comprises 2 to 20 lysine residues covalently linked to the polysaccharide through an eTEC spacer.

39. The glycoconjugate of claim 37, wherein the CRM_{197} comprises 4 to 16 lysine residues covalently linked to the polysaccharide through an eTEC spacer.

40. The glycoconjugate of any one of claims 23 to 39, wherein the saccharide : carrier protein ratio (w/w) is between 0.2 and 4.

41. The glycoconjugate of claim 40, wherein the saccharide : carrier protein ratio (w/w) is between 0.4 and 1.7.

42. The glycoconjugate of claim 37, wherein at least one linkage between CRM_{197} and saccharide occurs at every 25 saccharide repeat units of the saccharide.

43. The glycoconjugate of claim 37, wherein at least one linkage between CRM_{197} and saccharide occurs at every 15 saccharide repeat units of the saccharide.

44. The glycoconjugate of claim 37, wherein at least one linkage between CRM_{197} and saccharide occurs at every 10 saccharide repeat units of the saccharide.

45. The glycoconjugate of claim 37, wherein at least one linkage between CRM_{197} and saccharide occurs at every 4 saccharide repeat units of the saccharide.

46. The glycoconjugate of any one of claims 23 to 45, which comprises less than 15% free saccharide compared to the total amount of saccharide.

47. The glycoconjugate of any one of claims 23 to 46, having a molecular size distribution (K_d) of ≥35% at ≤ 0.3.

48. An immunogenic composition comprising the glycoconjugate of any one of claims 23 to 47 and a pharmaceutically acceptable excipient, carrier or diluent.

49. The immunogenic composition of claim 48, further comprising an additional antigen.

50. The immunogenic composition of claim 49, wherein the additional antigen comprises a protein antigen or a glycoconjugate of a capsular polysaccharide derived from *S. pneumonia*.

51. The immunogenic composition of claim 50, wherein the additional antigen comprises a glycoconjugate of a capsular polysaccharide selected from the group consisting of Pn-serotype 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F capsular polysaccharides.

52. The immunogenic composition of claim 49, wherein the additional antigen comprises a protein antigen or a glycoconjugate of a capsular polysaccharide derived from *N. meningitidis*.

53. The immunogenic composition of claim 52, wherein the additional antigen comprises a glycoconjugate of a capsular polysaccharide selected from the group consisting of serotype A, C, W135 and Y capsular polysaccharides.

54. The immunogenic composition of any one of claims 48 to 53, further comprising an adjuvant.

55. The immunogenic composition of claim 54, wherein the adjuvant is an aluminum-based adjuvant.

56. The immunogenic composition of claim 55, wherein the aluminum-based adjuvant is selected from the group consisting of aluminum phosphate, aluminum sulfate, and aluminum hydroxide.

57. An immunologically effective amount of an immunogenic composition of any one of claims 48 to 56 for use in preventing, treating or ameliorating a bacterial infection, disease or condition in a subject.

58. The use of claim 57, wherein the infection, disease or condition is associated with *S. pneumonia* bacteria and the glycoconjugate comprises a capsular polysaccharide selected from the group consisting of Pn-serotype 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F capsular polysaccharides.

59. The use of claim 57, wherein the infection, disease or condition is associated with *N. meningitidis* bacteria and the glycoconjugate comprises a capsular polysaccharide selected from the group consisting of serotype A, C, W135 and Y capsular polysaccharides.

60. An immunologically effective amount of an immunogenic composition of any one of claims 48 to 56 for use in inducing a protective immune response in a subject.

61. The use of claim 60, wherein the bacterial capsular polysaccharide is a capsular polysaccharide selected from the group consisting of Pn-serotype 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F capsular polysaccharides.

62. The use of claim 60, wherein the bacterial capsular polysaccharide is a capsular polysaccharide selected from the group consisting of serotype A, C, W135 and Y capsular polysaccharides.

Claims

1. A method of making a glycoconjugate comprising a saccharide conjugated to a carrier protein through a (2-((2-oxoethyl)thio)ethyl)carbamate (eTEC) spacer, comprising the steps of:

a) reacting a saccharide with 1,1'-carbonyl-di-(1,2,4-triazole) (CDT) or 1,1'-carbonyldiimidazole (CDI), in an organic solvent to produce an activated saccharide;

b) reacting the activated saccharide with cystamine or cysteamine or a salt thereof, to produce a thiolated saccharide;

c) reacting the thiolated saccharide with a reducing agent to produce an activated thiolated saccharide comprising one or more free sulfhydryl residues;

d) reacting the activated thiolated saccharide with an activated carrier protein comprising one or more α-haloacetamide groups, to produce a thiolated saccharide-carrier protein conjugate; and

e) reacting the thiolated saccharide-carrier protein conjugate with (i) a first capping reagent capable of capping unconjugated α-haloacetamide groups of the activated carrier protein; and/or (ii) a second capping reagent capable of capping unconjugated free sulfhydryl residues;

whereby an eTEC linked glycoconjugate is produced.

2. The method of claim 1, wherein the capping step e) comprises reacting the thiolated saccharide-carrier protein conjugate with (i) N-acetyl-L-cysteine as a first capping reagent, and/or (ii) iodoacetamide as a second capping reagent.

3. The method of claim 1 or 2, further comprising a step of compounding the saccharide by reaction with triazole or imidazole to provide a compounded saccharide, wherein the compounded saccharide is shell frozen, lyophilized and reconstituted in an organic solvent prior to step a).

4. The method of claim 1, 2 or 3, further comprising purification of the thiolated polysaccharide produced in step c), wherein the purification step comprises diafiltration.

5. The method of any one of claims 1 to 4, wherein the reducing agent in step c) is tris(-2-carboxyethyl)phosphine (TCEP), dithiothreitol (DTT) or mercaptoethanol.

6. The method of any one of claims 1 to 5, wherein the carrier protein is activated with the N-hydroxysuccinimide ester of bromoacetic acid (BAANS).

7. The method of any one of claims 1 to 6, wherein the method further comprises purification of the eTEC linked glycoconjugate by diafiltration.

8. The method of any one of claims 1 to 7, wherein the organic solvent in step a) is a polar aprotic solvent selected from the group consisting of dimethyl sulfoxide (DMSO), dimethylformamide (DMF), dimethylacetamide (DMA), *N*-methyl-2-pyrrolidone (NMP), acetonitrile, 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) and hexamethylphosphoramide (HMPA), or a mixture thereof.

9. The method of any one of claims 1 to 8, wherein the saccharide : carrier protein ratio (w/w) is between 0.2 and 4.

10. The method of claim 9, wherein the saccharide : carrier protein ratio (w/w) is between 0.4 and 1.7.

11. The method of any one of claims 1 to 10, wherein the saccharide is a capsular polysaccharide derived from *S. pneumoniae*.

12. The method of claim 11, wherein the capsular polysaccharide is selected from the group consisting of pneumococcal (Pn) serotype 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F capsular polysaccharides.

13. The method of claim 12, wherein the capsular polysaccharide is a Pn-serotype 33F capsular polysaccharide.

14. The method of claim 12, wherein the capsular polysaccharide is a Pn-serotype 22F capsular polysaccharide.

15. The method of claim 12, wherein the capsular polysaccharide is a Pn-serotype 10A capsular polysaccharide.

16. The method of claim 12, wherein the capsular polysaccharide is a Pn-serotype 11A capsular polysaccharide.

17. The method of any one of claims 1 to 10, wherein the saccharide is a capsular polysaccharide derived from *N. meningitidis*.

18. The glycoconjugate of claim 17, wherein the capsular polysaccharide is selected from the group consisting of meningococcal (Mn)-serotype A, C, W135 and Y capsular polysaccharides.

19. The method of any one of claims 1-18, wherein the carrier protein is CRM_{197}.

20. A glycoconjugate produced by the method of any one of claims 1-19.

21. An immunogenic composition comprising the glycoconjugate of claim 20 and a pharmaceutically acceptable excipient, carrier or diluent.

22. A glycoconjugate comprising a bacterial capsular polysaccharide conjugated to a carrier protein through an eTEC spacer, wherein the polysaccharide is covalently linked

to the eTEC spacer through a carbamate linkage, and wherein the carrier protein is covalently linked to the eTEC spacer through an amide linkage.

23. The glycoconjugate of claim 22, wherein the capsular polysaccharide is derived from *S. pneumoniae*.

24. The glycoconjugate of claim 23, wherein the capsular polysaccharide is selected from the group consisting of Pn-serotype 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F capsular polysaccharides.

25. The glycoconjugate of claim 24, wherein the capsular polysaccharide is a Pn-serotype 33F capsular polysaccharide.

26. The glycoconjugate of claim 24, wherein the capsular polysaccharide is a Pn-serotype 22F capsular polysaccharide.

27. The glycoconjugate of claim 24, wherein the capsular polysaccharide is a Pn-serotype 10A capsular polysaccharide.

28. The glycoconjugate of claim 24, wherein the capsular polysaccharide is a Pn-serotype 11A capsular polysaccharide.

29. The glycoconjugate of claim 22, wherein the capsular polysaccharide is derived from *N. meningitidis*.

30. The glycoconjugate of claim 29, wherein the capsular polysaccharide is selected from the group consisting of Mn-serotype A, C, W135 and Y capsular polysaccharides.

31. The glycoconjugate of any one of claims 22 to 30, wherein the polysaccharide has a molecular weight of between 10 kDa and 2,000 kDa.

32. The glycoconjugate of claim 31, wherein the polysaccharide has a molecular weight of between 50 kDa and 2,000 kDa.

33. The glycoconjugate of any one of claims 22 to 32, wherein the glycoconjugate has a molecular weight of between 50 kDa and 20,000 kDa.

34. The glycoconjugate of claim 33, wherein the glycoconjugate has a molecular weight of between 500 kDa and 10,000 kDa.

35. The glycoconjugate of any one of claims 22 to 34, wherein the polysaccharide has a degree of O-acetylation between 75-100%.

36. The glycoconjugate of any one of claims 22 to 35, wherein the carrier protein is CRM_{197}.

37. The glycoconjugate of claim 36, wherein the CRM_{197} comprises 2 to 20 lysine residues covalently linked to the polysaccharide through an eTEC spacer.

38. The glycoconjugate of claim 36, wherein the CRM_{197} comprises 4 to 16 lysine residues covalently linked to the polysaccharide through an eTEC spacer.

39. The glycoconjugate of any one of claims 22 to 38, wherein the saccharide : carrier protein ratio (w/w) is between 0.2 and 4.

40. The glycoconjugate of claim 39, wherein the saccharide : carrier protein ratio (w/w) is between 0.4 and 1.7.

41. The glycoconjugate of claim 36, wherein at least one linkage between CRM_{197} and saccharide occurs at every 25 saccharide repeat units of the saccharide.

42. The glycoconjugate of claim 36, wherein at least one linkage between CRM_{197} and saccharide occurs at every 15 saccharide repeat units of the saccharide.

43. The glycoconjugate of claim 36, wherein at least one linkage between CRM_{197} and saccharide occurs at every 10 saccharide repeat units of the saccharide.

44. The glycoconjugate of claim 36, wherein at least one linkage between CRM_{197} and saccharide occurs at every 4 saccharide repeat units of the saccharide.

45. The glycoconjugate of any one of claims 22 to 44, which comprises less than 15% free saccharide compared to the total amount of saccharide.

46. The glycoconjugate of any one of claims 22 to 45, having a molecular size distribution (K_d) of ≥35% at ≤ 0.3.

47. An immunogenic composition comprising the glycoconjugate of any one of claims 22 to 46 and a pharmaceutically acceptable excipient, carrier or diluent.

48. The immunogenic composition of claim 47, further comprising an additional antigen.

49. The immunogenic composition of claim 48, wherein the additional antigen comprises a protein antigen or a glycoconjugate of a capsular polysaccharide derived from *S. pneumonia*.

50. The immunogenic composition of claim 49, wherein the additional antigen comprises a glycoconjugate of a capsular polysaccharide selected from the group consisting of Pn-serotype 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F capsular polysaccharides.

51. The immunogenic composition of claim 48, wherein the additional antigen comprises a protein antigen or a glycoconjugate of a capsular polysaccharide derived from *N. meningitidis*.

52. The immunogenic composition of claim 51, wherein the additional antigen comprises a glycoconjugate of a capsular polysaccharide selected from the group consisting of serotype A, C, W135 and Y capsular polysaccharides.

53. The immunogenic composition of any one of claims 47 to 52, further comprising an adjuvant.

54. The immunogenic composition of claim 53, wherein the adjuvant is an aluminum-based adjuvant.

55. The immunogenic composition of claim 54, wherein the aluminum-based adjuvant is selected from the group consisting of aluminum phosphate, aluminum sulfate, and aluminum hydroxide.

56. An immunologically effective amount of an immunogenic composition of any one of claims 47 to 55 for use in preventing, treating or ameliorating a bacterial infection, disease or condition in a subject.

57. The use of claim 56, wherein the infection, disease or condition is associated with *S. pneumonia* bacteria and the glycoconjugate comprises a capsular polysaccharide selected from the group consisting of Pn-serotype 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F capsular polysaccharides.

58. The use of claim 56, wherein the infection, disease or condition is associated with *N. meningitidis* bacteria and the glycoconjugate comprises a capsular polysaccharide selected from the group consisting of serotype A, C, W135 and Y capsular polysaccharides.

59. An immunologically effective amount of an immunogenic composition of any one of claims 47 to 55 for use in inducing a protective immune response in a subject.

60. The use of claim 59, wherein the bacterial capsular polysaccharide is a capsular polysaccharide selected from the group consisting of Pn-serotype 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F capsular polysaccharides.

61. The use of claim 59, wherein the bacterial capsular polysaccharide is a capsular polysaccharide selected from the group consisting of serotype A, C, W135 and Y capsular polysaccharides.